

of microscopic diversity and reduces the well-known taxonomic bias in favour of easily identifiable taxa and against cryptic biodiversity. By combining morphological and molecular biodiversity in a single taxonomic backbone using Linnaean names, OTU codes, and molecular occurrence data from BOLD, UNITE, EMBL-EBI, and SILVA, the GBIF network will address major spatial, temporal, and taxonomic biases while supporting scientific efforts to understand functional biodiversity. GBIF promotes open data while protecting data provenance through data citation, and new API-based tools will further scale up cross-platform data integration and data indexing. These efforts address the growing need for easily accessible molecular biodiversity evidence.

Effects of climate-induced tree-dieback on freshwater and Malaise trap communities in the Bavarian Forest National Park

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Background: Mountain forest ecosystems are under increasing pressures. Rising global temperatures lead to increased frequency of droughts and pathogen outbreaks, which in turn lead to drastic changes in forest structure and widespread die-off of key tree species. How these forest die-offs affect arthropod communities and biodiversity overall is not well understood, and current biomonitoring methods are inadequate for large-scale quality assessments. The Bavarian Forest National Park has a long history of research on mountain forests, in particular on spruce (*Picea abies*), and an ongoing insect monitoring program. **Results:** We used Malaise traps to sample invertebrates in 30 plots within three different forest habitats (intact, naturally disturbed, salvage-logged) along an elevational gradient over four months in summer 2017 for a total of 240 samples. We used kick-netting to sample invertebrates in 30 stream sites within the same forest habitats. DNA was extracted from subsamples and metabarcoding libraries (313 bp COI) were sequenced with Illumina MiSeq. We used replicate PCRs ($n = 3$) and twin-tagging, together with a mock community and negative controls, in order to carry out stringent filtering. To date, >4000 operational taxonomic units (OTUs) have been identified for the Malaise traps, with >70% belonging to Diptera or Hymenoptera and Ichneumonidae being the most diverse family (>800 OTUs). **Significance:** The project is part of a larger effort that combines ecological and socio-economic approaches to understand mountain forest die-back and its implications for biodiversity. Our specific goal is to develop a fast, repeatable, and reliable method for biomonitoring of terrestrial and aquatic invertebrates in mountain forests that will help practitioners such as national parks and forest owners. We hope to further our knowledge of methodological processes and techniques to make monitoring results in the future more comparable and standardized, and to gain additional insights into the ecology of invertebrate communities in mountain forest habitats.

Multiplexed chloroplast and nuclear marker sets for differentiation of 19 relevant poplar species for breeding

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Background: The genus *Populus* includes about 30 species classified in six sections, where some are cross-compatible even between sections. Therefore, many naturally and additionally, due to breeding programs, artificially produced hybrids exist, often without any information about involved species. Due to the very high variability of *Populus* hybrids, species identification using morphological characters is sometimes difficult. For this reason, we combined classical barcoding markers with newly designed chloroplast and nuclear markers with the aim to develop sets of

markers for the differentiation of the 19 most widely used poplar species. **Results:** We used 1–32 individuals per species for sequencing of a total of nine chloroplast and four nuclear regions. Overall, we found species-specific SNPs or indels for 14 of the 19 species in chloroplast and 17 out of 19 species in nuclear regions. Nucleotide diversity in the analysed regions varied among species and was highest for the three species of the section *Populus* (*P. alba*, *P. tremula*, *P. tremuloides*) followed by the Aigeiros species *P. nigra*. We developed methods to identify species by either species-specific nucleotide variations or, without initial information for the species, by using markers either in a step-wise procedure of exclusion or in a multiplexed marker set. The two species *P. koreana* and *P. ussuriensis* are not distinguishable by applying both procedures. **Significance:** Hybrids between various *Populus* species belonging to the same or different sections are commonly used in short rotation coppices for biomass production because of their superior growth and advanced resistance traits. We present a comprehensive study on the identification of a high number of *Populus* species that, to our knowledge, have not been performed across such a wide range and with feasibility in any laboratory.

Barcoding Canada's ecozones: past progress, future steps

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DNA barcode reference libraries are the foundation for scalable, high-resolution biosurveillance systems. Over the past 15 years, 2.1 million Canadian specimens have been barcoded, providing coverage for 77 391 BINs and 31 253 named species. Most of these records (95.2%) derive from terrestrial settings; just 4.8% derive from the marine environment although it comprises more than a third of Canadian territory. The differences are even more profound when one considers coverage from an ecozone perspective. Coverage for the 15 terrestrial ecozones averages 129 909 records but ranges from 2463 records (Taiga Shield) to 582 663 (Mixed Wood Plain). Coverage for the 12 marine ecozones is far lower, averaging 5591 records and ranging from a low of just 58 (Arctic Archipelago) to a high of 22 617 (Strait of Georgia). The average number of records on an areal basis differs by more than an order of magnitude between marine and terrestrial settings (1.2 vs. 19.5 records per 100 km²). When viewed at a species level, coverage for marine phyla ranges from near zero for bryozoans, ctenophores, and nematodes to 56.6% for arthropods. Moreover, many species in the oceans await discovery as metabarcoding studies on marine benthos indicate high diversity. Studies are now underway to strengthen coverage in marine environments through studies on hyperdiverse groups such as harpacticoid copepods and nematodes that have seen little prior investigation.

Identification of tree species in wood composite products by DNA barcoding

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Background: In Europe and in the USA, the detailed labeling of imported wood is an attempt to control illegal logging. Finding out the mixture of species in wood composite products poses a great challenge due to the use of different wood species in one product; the exposure of wood to chemicals, heat, and pressure during the production process; and the restricted applicability of wood anatomical methods. **Results:** Wood composite products often consist of wood from several tree species, which may belong to the group of angiosperms as well as to the gymnosperms. We are developing a set of genetic markers for the differentiation of kinds of frequently used wood on different taxonomic levels. The combination enables the development of an application protocol for an individualized and minimized marker set for each genus and species of interest. The marker development is based on a genome-wide identification of SNPs and InDels in chloroplast and mitochondrial genomes. For the differ-

entiation of angio- and gymnosperms, a CAPS marker was developed based on a SNP identified in the mitochondrial COX1 gene, which is used for species identification in many animal groups. Additionally, the absence or presence of certain *ndh* genes in chloroplast genomes will be used to confirm these results. At the genus level, e.g., in the family of Betulaceae, species belonging to the genus *Betula* can be separated from all others by only one CAPS marker in the *matK* gene, a formerly suggested chloroplast barcoding region. In a next step, we will also include gene distance and intron length information in the search for new markers. **Significance:** Illegal logging is the most profitable natural resource crime in the world. Thus, the DNA-based analysis of wood samples plays an important role as a law-enforcement tool in the determination of botanical species and geographic origin.

Developing PCR-RFLP and SNP-based markers for determining cytoplasmic male sterile factors in the genus *Amaranthus* (Amaranthaceae)

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Background: *Amaranthus* L. is an herbaceous plant comprising approximately 70 species, with three subgenera, which contains both cultivated and wild types. The cultivated species are used for food grains, leafy vegetables, potential forages, and ornamentals. Genetic diversity analysis in amaranths is important for the development of a core set of germplasm from widely diverse populations and for the effective utilization of plant genetic resources. Cytoplasmic male sterility (CMS) is one of the most important traits in crop breeding, which has been used for commercial seed production by F1 hybrid cultivars of *Amaranthus* species. Our aim is to develop a polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) marker to distinguish male-fertile (N) and male-sterile (S) cytoplasm in *Amaranthus* species by examining the distribution of the haplotypes in diverse breeding lines, cultivars, and wild. **Results:** The PCR-RFLP marker was located in a chloroplast *psbA* gene amplicon. Digesting the amplicons from different cytoplasm (either N/S)-containing varieties with restriction enzyme revealed and distinguished the N and S with functional and substitution cytoplasmic site. The developed PCR-RFLP marker was validated for cytoplasmic male sterile factors in 15 samples belonging to the wild and cultivated cultivars, which showed CMS-specific sequence-characterized amplified region (SCAR) marker. Moreover, the PCR-RFLP marker can identify N- or S-cytoplasm in DNA sample mixtures in which they are 10-fold less, indicating that use of the marker has diagnostic precision. **Significance:** We also confirmed the efficacy of the SNP detected in the *psbA* gene for high-throughput discrimination of CMS factors using real-time PCR. This approach is useful for the identification of CMS factors in large *Amaranthus* breeding populations and also for facilitating the crop's improvement.

DNA barcoding of Norwegian forest Oribatida — preliminary results reveal several taxonomic problems

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Background: Oribatid mites (Sarcoptiformes) are a very common, abundant, and diverse group with important roles in ecosystems and are also known as good bioindicators. Worldwide, Oribatida (excluding Astigmata) are represented by 9400 named species and 161 families. The Norwegian checklist mentions 244 species. BOLD includes over 43 000 specimen records, 40 000 sequences, and about 50% of all world-known families represented. The public data include 27 000 sequences in 2300 BINs. However, the number of named species is low, seriously limiting the use of this database for the identification purposes. The public BOLD database includes 185 named oribatid species only (i.e., 2% of all oribatid species known worldwide). **Results:** During ongoing studies on the diversity of Oribatida in Norwegian broad-

leaved forests, 272 sequences (80% of barcoded specimens) in 91 BINs were obtained, representing 86 species, 53 genera, and 34 families. Only 20% of the species were represented in BOLD. Most species were monophyletic, with limited mitochondrial DNA variation. However, in about 10% of them the variation was 5%–10%, and in a further 10% it exceeded 10%. High mitochondrial DNA variation was noticed in species with a semi-cosmopolitan distribution and in some less-studied taxa. **Significance:** These preliminary results reveal 17 taxa that require special attention, as they might represent species complexes and include cryptic species. We also contributed 60 named species of Oribatida to the BOLD database.

Molecular diversity in the monotypic catfish genus *Mastiglanis* (Bockmann, 1994)

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Background: Catfish of the Family Heptapteridae are small-to-medium sized benthic omnivores, generally found in smaller streams, and endemic to the Neotropical region. Many heptapterids present systematic challenges because of the morphological similarity between species and even genera. The monotypic genus *Mastiglanis* is frequently identified incorrectly as *Imparfinis* in regional ecological surveys because of the similarity in terms of long barbels, a deeply forked tail, and limited pigmentation of the body. The genera differ most obviously in terms of pectoral fin ray lengths, and this character can often be lost during rough sample handling or naturally, prior to collection, through the action of predators from which the individual has narrowly escaped. We therefore applied a DNA barcoding approach to assess the potential existence of divergent lineages that may represent overlooked or cryptic species. We bidirectionally sequenced the COI-5P fragment (~650 bp) commonly used in vertebrate taxa and shown to be useful for species delimitation in freshwater and marine fish taxa. **Results:** Sequences were produced for 17 individuals identified as *Mastiglanis asopos* from nine different localities in the eastern Amazon—Pará state, Brazil. These sequences were combined with public data for other species in the family to produce a phylogenetic tree. These data were then analyzed using both threshold and coalescent species delimitation techniques. The overall phylogeny confirmed that *Imparfinis* and *Mastiglanis* are distinct evolutionary lineages, but showed that the samples identified as *Mastiglanis* do not form a monophyletic group. They represent two very divergent clades (probably separate genera), one of which contains five distinct lineages. **Significance:** The genus *Mastiglanis* is described to have a very large distribution across cis-Andean drainages of South America. As such, further sampling is required to determine the geographic distributions of each of its lineages and incorporate the information into integrated systematic results.

Spatio-temporal dynamics of lotic eDNA-derived metacommunities

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Background: Accurately measuring biodiversity is essential for ecology, molecular ecology, and environmental monitoring. Recently, en-